Antihypertensive and vasorelaxant effects of ethanol extract of stem barks from *Zanthoxylum rhoifolium* Lam. in rats

Edson Santos Ferreira-Filho\(^a\), Daniel Dias Rufino Arcanjo\(^*\), Lucas Henrique Porfirio Moura\(^a\), José Coursa da Silva-Filho\(^a\), Emanuel Tenório Paulino\(^b\), Éurica Adélia Nogueira Ribeiro\(^b\), Mariana Helena Chaves\(^c\), Rita de Cássia Meneses Oliveira\(^a\)& Aldeídia Pereira de Oliveira\(^a\)

\(^a\)Medicinal Plants Research Center, Federal University of Piauí, Av. Nossa Senhora de Fátima s/n, SG-15, 64049-550, Teresina, PI, Brazil

\(^b\)Cardiovascular Pharmacology Laboratory, Federal University of Alagoas, Brazil.

\(^c\)Department of Chemistry, Federal University of Piauí, Teresina, PI, Brazil.

Received 25 June 2012; revised 15 May 2013

Administration of ethanol extract of stem bark from *Z. rhoifolium* (EEtOH-ZR) induced hypotension associated with a dual effect in heart rate in normotensive rats. This response was highlighted in spontaneously hypertensive rats (SHR).

In rat superior mesenteric artery rings, the cumulative addition of EEtOH-ZR (0.1–750 µg/mL) on a phenylephrine-induced pre-contraction (10\(^{-5}\) M) promoted a vasorelaxant effect by a concentration-dependent manner and independent of vascular endothelium. A similar effect was obtained on KCl-induced pre-contractions (80 mM). EEtOH-ZR attenuated contractions induced by cumulative addition of CaCl\(_2\) (10\(^{-6}\)–3 × 10\(^{-2}\) M) in depolarizing medium without Ca\(^{2+}\) only at 500 or 750 µg/mL. Likewise, on S-(-)-Bay K 8644-induced pre-contractions (10\(^{-7}\) M), the EEtOH-ZR-induced vasorelaxation effect was attenuated. EEtOH-ZR (27, 81, 243 or 500 µg/mL) inhibited contractions induced by cumulative addition of phenylephrine (10\(^{-9}\)–10\(^{-5}\) M) in a Ca\(^{2+}\)-free medium.

The involvement of K\(^+\) channels was evaluated by tetraethylammonium (3 mM); the EEtOH-ZR-induced vasorelaxation was not attenuated.

Thus, calcium influx blockade through voltage-operated calcium channels (Ca\(_{V_L}\)) and inhibition of calcium release from intracellular stores are probably underlying EEtOH-ZR-induced cardiovascular effects.

**Keywords:** Anti-hypertensive, Cardiovascular, Hypertension, Vasorelaxation, *Zanthoxylum rhoifolium*

Hypertension is a major public health problem worldwide and, when untreated, predisposes to cardiovascular morbidity and premature death\(^1\),\(^2\). Effective treatment of hypertension reduces complications and improves life expectancy. However, the difficulty of providing access to antihypertensive medications in developing countries justifies the search for new antihypertensive agents of natural origin, especially in countries like Brazil, where there is a large potential for the development of herbal preparations\(^3\). Further, natural products represent an extremely valuable source for production of new chemical entities for the treatment of untreated diseases, since they represent privileged structures selected by evolutionary mechanisms over a period of millions of years\(^4\).

The genus *Zanthoxylum* (Rutaceae) comprises more than 200 species distributed worldwide and several species are used in folk medicine to treat cardiovascular diseases\(^5\)–\(^8\). *Zanthoxylum rhoifolium* Lam. (Rutacae), popularly known as “mamica-de-cadela” or “mamica-de-porca”\(^9\), is a native tree of Brazil, which grows up to 12 m found on the forest and savannah areas in almost all south-central Brazil\(^10\). It has been popularly used in inflammatory, microbial, cancerous and malaria processes\(^11\). The stem bark is used in traditional medicine as teas or infusions as antiinflammatory, antispasmodic, analgesic, sudorific, antifungal and diuretic\(^12\).

From methanol extract of the bark of *Z. rhoifolium*, three benzophenanthridine alkaloids have been isolated: dihydronitidine, oxydimidine and zanthoxiline, together with the known compounds, dihydronitidine, 6-oxydimidine and skimmianine\(^13\). Also, analyses of the essential oil from *Z. rhoifolium* leaves showed that among its numerous constituents, there are some components described as cytotoxic substances against tumoral cells, such as β-caryophyllene, β-elemene, δ-elemene, α-humulene\(^11\).
The phytochemical screening of ethanol extract of stem barks from Z. rhoifolium by thin-layer silica gel chromatography, using specific spray reagents, suggested the presence of isoprene nature substances (triterpenes and steroids), as well as flavonoids and alkaloids. The presence of triterpenes was confirmed by the isolation and identification of lupeol, a pentacyclic triterpenoid obtained from hexane fraction of partition and determined by $^1$H and $^{13}$C NMR spectroscopic analyses. Besides, the ethanol extract elicited antimicrobial and gastroprotective effects in models of chemical nociception and gastric ulcers, respectively, in rodents$^{14,15}$.

The effects of Z. rhoifolium on cardiovascular system, especially in hemodynamic parameters in vivo and its mechanisms of vasorelaxation still remain unknown. Therefore, the aim of this study is to characterize the vasorelaxant and the antihypertensive effects elicited by the ethanolic extract from stem barks of Z. rhoifolium Lam. (EEtOH-ZR) and also contribute towards the pharmacological knowledge about this plant.

Materials and Methods

Plant material identification and extraction—Stem barks of Z. rhoifolium were collected in 2005 from Pedro II city, Piauí state, Brazil. The voucher specimen (TEPB 13870) was identified by a botanist and deposited at Graziela Barroso Herbarium of Federal University of Piauí (UFPI). EEtOH-ZR was obtained according to Pereira et al$^{14}$. Dried and powdered barks of Z. rhoifolium (1.0 kg) were exhaustively extracted at room temperature with ethanol. The solvent was removed by evaporation under reduced pressure using a Hedolph Rotary Evaporator to yield the ethanolic extract (85.0 g, 8.5%). Prior to the experiments, EEtOH-ZR was dissolved in a mixture of distilled water and 3% cremophor solution and kept at -4 ºC.

Animals—Male Wistar rats (Rattus norvegicus, 8–10 weeks old, 250–350 g) were used. For the evaluation of the antihypertensive effect, male Wistar rats and Spontaneously Hypertensive Rats (SHR) were used. The animals were kept in well-ventilated cages at controlled temperature (24±2 ºC) and under controlled light cycles (12:12 h L:D). They were maintained on normal laboratory chow and water ad libitum. All experiments were approved by Committee of Ethics for Animal Research of Federal University of Piauí, Brazil (CEEA No. 029/09). The protocols agree with the guidelines of the National Institute of Health (NIH Publication 85-23, 1985) for laboratory animal care and use.

Drugs—The following drugs were used: L-phenylephrine hydrochloride (PHE), acetylcholine hydrochloride (ACh), tetraethylammonium (TEA), S-(–)-Bay K 8644 (Sigma Chemical Co., Saint Louis, MO, USA), heparin sodium salt (Roche Brazil, São Paulo, Brazil) and sodium thiopental (Cristalia, Butantã, Brazil). All the stock solutions were prepared in distilled water. S-(–)-Bay K 8644 was previously dissolved in ethanol.

Solutions—The composition of the Tyrode’s solution used was (in mM): NaCl, 158.3; KCl, 4.0; CaCl$_2$-2H$_2$O, 2.0; MgCl$_2$-6H$_2$O, 1.05; NaHCO$_3$, 10.0; NaH$_2$PO$_4$·H$_2$O, 0.42; and glucose, 5.6, all from Sigma Chemical Co., Saint Louis, MO, USA$^{16}$. Depolarizing Tyrode solutions with KCl 20, 60 or 80 mM were prepared by an equimolar replacement of Na$^+$ for K$^+$.$^{17}$ In nominally without Ca$^{2+}$ depolarizing solution, CaCl$_2$ was not added, and in Ca$^{2+}$-free solution, CaCl$_2$ was omitted and 1 mM ethylene-diamine-tetraacetic acid (EDTA), was added$^{18}$.

Effects of EEtOH-ZR on cardiovascular parameters in non-anaesthetized rats—The procedures for arterial pressure (AP) and heart rate (HR) recordings were done as previously described.$^{19}$ Briefly, under sodium thiopental anaesthesia (45 mg/kg, ip), rats were fitted with polyethylene catheters inserted into the lower abdominal aorta and inferior vena cava through left femoral artery and vein, respectively. Both catheters were filled with heparinized saline, tunnelled subcutaneously, exteriorized and sutured at the dorsal surface of the neck. After 24 h of surgery, experiments were performed in conscious rats. The arterial catheter was connected to a precalibrated pressure transducer, which was connected to an amplifier–recorder (AVS Projeto, São Paulo, Brazil). Beat-to-beat time waveforms were generated and processed offline. For each cardiac cycle, the software calculated mean arterial pressure (MAP) and pulse interval (PI), used to derive HR.

Evaluation of EEtOH-ZR-induced vasorelaxant effects

Preparation of superior mesenteric artery rings—Male Wistar rats were euthanized by cervical
concentration–response curves for CaCl
endothelium-denuded rings was assessed. Cumulative
denuded preparations.
or KCl-induced pre-contractions in endothelium-
EEtOH-ZR on CaCl$_2$
expressed as EC$_{50}$ in the rings was also examined. The results were
stabilized, approximately 30 minutes later. Then, a
500 and 750
added cumulatively (0.1, 0.3, 1, 3, 9, 27, 81, 243, 500 and 750 µg/mL)
were obtained in endothelium-denuded rings exposed to
KCl before and after pre-incubation, separately with
EEtOH-ZR (81, 243, 500 or 750 µg/mL) for
8644 (10$^{-6}$ M) were also designed. Curves were
obtained in endothelium-denuded rings by a stepwise
increase in the concentration of PHE, in the absence
of KCl. PHE- or KCl-induced pre-contractions (80 mM) in
derived from aorta ring preparations were pre-incubated with a
depolarizing Tyrode solution of KCl 20 mM for 20
min, a procedure performed to obtain a better
response for the contractile agent. Then, S(−)-Bay K
8644 (10$^{-5}$ M), a Ca$_{V1.2}$ channels activator was added to
induce a sustained contraction, and EEtOH-ZR
(0.1, 0.3, 1, 3, 9, 27, 81, 243, 500 and 750 µg/mL)
was cumulative added on this contractile tonus
and results were considered significant when
contractions in endothelium-denuded preparations.
Effect of EEtOH-ZR on cumulative PHE-induced
contractions—Cumulative-response curves for PHE
(10$^{-6}$–10$^{-5}$ M) were also designed. Curves were
obtained in endothelium-denuded rings by stepwise
increase in the concentration of PHE, in the absence
(control) or after a 30 min incubation period with
EEtOH-ZR (27, 81, 243 and 500 µg/mL). The results
were expressed as percentages of the maximal
response for only PHE-induced response, and curves
were statistically compared
Effect of EEtOH-ZR on PHE-sensitive calcium intracellular stores—The effect of EEtOH-ZR on
PHE-sensitive calcium intracellular stores was assessed. The transient contractions were obtained in
endothelium-denuded rings by PHE (10$^{-5}$ M) in Ca$^{2+}$-
free Tyrode solution before and after incubation of
EEtOH-ZR (27, 81, 243 and 500 µg/mL) for 3 min.
The results were expressed as percentages of the
response induced by PHE alone.
Effect of K$^+$ channels involvement in EEtOH-ZR-
induced vasorelaxant response—To investigate the possible involvement of potassium channels in
EEtOH-ZR-induced vasorelaxation, endothelium-
denuded rings were pre-incubated with TEA (3 mM),
a non-specific K$^+$ channel blocker, for 30 min. After
stabilization of PHE-induced tonic vasoconstriction,
cumulative concentrations of EEtOH-ZR were added
to the organ bath. The results were expressed as EC$_{50}$
values and compared with PHE-induced pre-
contractions in endothelium-denuded preparations.
Statistical analysis—All values were expressed as
mean ± SE. Curves and EC$_{50}$ values were
obtained by nonlinear regression. Student’s t-test
and ANOVA-One-way or Two-way followed by
Bonferroni post-test were used in the data analysis
and results were considered significant when
P<0.05. All analysis was performed using GraphPad Prism
5.0 (GraphPad Software, San Diego, CA, USA).
Results

Effect of EEtOH-ZR on mean arterial pressure (MAP) and heart rate (HR) in non-anaesthetized rats—

Systemic haemodynamic changes induced by EEtOH-ZR were investigated in conscious normotensive and spontaneously hypertensive rats (SHR). In normotensive rats, the baseline values of MAP and HR were 122±5 mmHg and 300±10 bpm, respectively. Administration of EEtOH-ZR at doses 0.5, 1.0, 5.0 and 10.0 mg/kg (iv) induced hypotension by 17.4±3.5, 15.8±2.3 and 11.3±1.3%, respectively (expressed as percentage of baseline values) that was associated with a dual effect in HR. Higher doses (20.0 and 30.0 mg/kg, iv) were able to elicit hypertension and tachycardia.

In SHR, the baseline values of MAP and HR were 175±10 mmHg and 400±10 bpm, respectively. Administration of EEtOH-ZR at doses 0.5, 1.0 and 5.0 mg/kg (iv) induced hypotension by 15.0±4.7%, 25.3±6.3% and 22.1±2.1%, respectively that was also associated with a dual effect in HR. Higher doses (10.0, 20.0 and 30.0 mg/kg, iv) also elicited hypertension and tachycardia (Fig. 1).

Effect of EEtOH-ZR on PHE- or KCl-induced contractions—In rat endothelium-intact isolated superior mesenteric rings, EEtOH-ZR induced a vasorelaxant response on PHE-induced pre-contraction (Fig. 2; EC$_{50}$=240.0±19.91 µg/mL), which was attenuated neither after removal of the vascular endothelium (EC$_{50}$=301.82±38.46 µg/mL) nor on KCl-induced pre-contractions in endothelium-denuded preparations (Fig. 3; EC$_{50}$=262.68±6.28 µg/mL).

Effects of EEtOH-ZR on cumulative CaCl$_2$-induced contractions—In a nominally without Ca$_{2+}$ depolarizing Tyrode solution, EEtOH-ZR only reduced the CaCl$_2$-induced maximal contractile response (E$_{max}$) at 500 or 750 µg/mL (Fig. 4).

Effects of EEtOH-ZR on S-(-)-Bay K 8644-induced pre-contractions—In endothelium-denuded rat superior mesenteric rings, increasing concentrations of EEtOH-ZR (0.1–750 µg/mL) induced a vasorelaxant response on S-(-)-Bay K 8644 (10$^{-7}$ M)-induced pre-contraction. However, the concentration-response curve obtained was rightward shifted compared with EEtOH-ZR vasorelaxant effect on KCl-induced pre-contraction (Fig. 5). The, EEtOH-ZR-induced vasorelaxant effect was then attenuated.

Effects of EEtOH-ZR on cumulative PHE-induced contractions—The pre-incubation of EEtOH-ZR for 30 min was able to inhibit in a concentration-dependent manner the maximal contractile response (E$_{max}$) induced by PHE (10$^{-9}$ – 10$^{-5}$ M) on endothelium-denuded rings. The concentration-response curve was significantly rightward shifted with inhibition of maximal effect (Fig. 6).

Effects of EEtOH-ZR on PHE-sensitive calcium intracellular stores—In Ca$^{2+}$-free depolarizing Tyrode solution, EEtOH-ZR promoted a concentration-dependent inhibition of PHE-induced contractions on rat mesenteric artery rings (Fig. 7; E$_{max}$ values: 27 µg/mL=78.48±7.71; 81 µg/mL=54.46±8.22; 243 µg/mL=25.51±4.06 and 500 µg/mL=11.58±4.50%).

Participation of K$^+$ channels in EEtOH-ZR-induced vasorelaxant response—In rat endothelium-denuded isolated superior mesenteric rings, pretreatment by TEA (3 mM) did not attenuated the EEtOH-ZR-induced vasorelaxation, and the concentration-response curve was leftward shifted (Fig. 8), indicating the non-participation of the K$^+$ channels in this response.
Fig. 2—Vasorelaxant response induced by EEtOH-ZR (0.1–750 µg/mL) on PHE-induced pre-contractions in endothelium-intact or -denuded rat superior mesenteric artery isolated rings. Values are mean±SE from 5 experiments.

Fig. 3—Vasorelaxant response induced by EEtOH-ZR (0.1–750 µg/mL) on PHE (10⁻⁵ M) or KCl (80 mM) induced pre-contractions in endothelium-denuded rat mesenteric artery rings. Values are mean±SE from 5 experiments.

Fig. 4—Concentration-response curves to EEtOH-ZR (81, 243, 500 or 750 µg/mL) on CaCl₂-induced contractions (10⁻⁶–3×10⁻³ M) in endothelium-denuded rat mesenteric artery rings in nominally without Ca²⁺ depolarizing Tyrode solution. Values are mean±SE from 5 experiments. P values: ** <0.01 and *** <0.001 vs. control.

Fig. 5—Vasorelaxant response induced by EEtOH-ZR (0.1–750 µg/mL) on KCl (80 mM) or S-(−)-Bay K 8644 (10⁻⁷ M) induced pre-contractions in endothelium-denuded rat mesenteric artery rings. Values are mean±SE from 5 experiments. P value: **<0.01 vs. KCl 80 mM.
Fig. 6—Concentration-response curves to EEtOH-ZR (27, 81, 243 or 500 µg/mL) on PHE-induced contractions (10^{-9}–10^{-5} M) in endothelium-denuded rat mesenteric artery rings. Values are mean±SE from 5 experiments. P values: *<0.05 and ***<0.001 vs. control.

Fig. 7—Inhibitory effect of EEtOH-ZR (27, 81, 243 or 500 µg/mL) on PHE 10^{-5} M-induced transient contractions in endothelium-denuded rat mesenteric artery in Ca^{2+}-free depolarizing Tyrode solution. Values are mean±SE from 5 experiments. P values: **<0.01, ***<0.001 vs. control.

Discussion
The present study shows that EEtOH-ZR intravenously administrated in conscious normotensive and SHR rats evoked biphasic changes on mean arterial pressure and dual effects on heart rate (Fig. 1). At lower doses, EEtOH-ZR was capable of inducing both hypotensive and antihypertensive effects, which come with dual response on heart rate: the tachycardia observed for doses of 1.0 mg/kg (normotensive) and 5.0 mg/kg (SHR) may probably due to baroreflex response after the direct hypotensive effects of EEtOH-ZR. Otherwise, the bradycardia observed for 5.0 mg/kg (normotensive) and 10.0 mg/kg (SHR) doses may probably due to decreased cardiac inotropism, which is shown by the majority of the most common antihypertensive and vasodilator drugs. Regarding the highest doses tested, it is possible that the increase in mean arterial pressure values is due to a cardiac action of the extract, implying a positive inotropic effect which eventually led to an increase of cardiac output and subsequently overcame the effect on the peripheral vascular resistance. A particularly interesting aspect was that similar responses were observed for both normotensive and spontaneously hypertensive rats, with different potencies: the behaviour of cardiovascular parameters of a dose of EEtOH-ZR in normotensive animals can be predicted by observing the effects of the immediately lower dose on these parameters in SHR, which can be explained by different functioning of the physiology of these animals when it comes to expression of receptors, ion channels and contractile proteins.
These results are consistent with the cardiovascular effect reported for several species from genus *Zanthoxylum*. In isolated rabbit aortic rings, the crude extract of *Z. armatum* exhibited cardiodepressant and vasodilator effect against phenylephrine and K\(^+\)-induced contractions. Likewise, *Z. schinifolium* elicits an increase in atrial dynamics, cAMP efflux, and decrease in ANP secretion, suggesting a cardioprotective effect by activation of β₁-adrenoceptor and cAMP-PKA-Ca\(^{2+}\)-signaling pathway in rabbit atrium.

Moreover, EEtOH-ZR induced a vasorelaxation on pre-contracted rat mesenteric artery rings in a concentration-dependent, which may partially justify its effect on blood pressure and heart rate. It is well known that the endothelium is an important regulator of the vascular tone by releasing endothelium-derived relaxing factors. In order to investigate its participation in the vasorelaxant effect induced by EEtOH-ZR, experiments were performed in absence of functional endothelium. This vasorelaxant effect was not decreased in endothelium-denuded preparations, suggesting an endothelium-independent effect and a direct action on vascular smooth muscle. The choice of this artery is due to its substantial contribution to the regulation of systemic circulation and also reflects the variation of vascular resistance in circulation.

For an endothelium-independent vasodilator effect, several mechanisms may be involved, including: blockade of extracellular Ca\(^{2+}\) influx through transmembrane Ca\(^{2+}\) channels; inhibition of agonist-mediated release of Ca\(^{2+}\) from intracellular stores; opening of K\(^+\) channels; inhibition of the contractile apparatus; among others. Since EEtOH-ZR also produced relaxation of isolated arteries pre-contracted by depolarization with KCl, which elicits contraction by allowing the influx of extracellular Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels, the hypothesis that EEtOH-ZR can inhibit vasoconstriction induced by extracellular Ca\(^{2+}\) influx should be previously considered. To confirm this observation, CaCl\(_2\)-induced concentration-response curves before and after EEtOH-ZR addition were obtained in a nominally without Ca\(^{2+}\) depolarizing Tyrode solution (KCl 60 mM). However, EEtOH-ZR induced an inhibition of CaCl\(_2\)-induced contractions only at 500 or 750 µg/mL, indicating a possible effect on voltage-operated calcium channels only in the higher concentrations.

Vascular smooth muscle cells (VSMCs) use calcium as a molecular signal for multiple functions. In the majority of VSMCs, excitation-contraction coupling is maintained by L-type Ca\(^{2+}\) channels (Ca\(_V\)L), regulating the calcium influx leading to vasoconstriction. The main voltage-operated calcium channel found in vascular smooth muscle is the Ca\(_V\)1.2, a Ca\(_V\)L subtype present in various smooth muscle cells, including VSMCs. Thus, concentration-response curves to EEtOH-ZR were performed after precontraction with S-(-)-Bay K 8644, a Ca\(_V\)1.2 activator. EEtOH-ZR promoted vasorelaxation of endothelium-denuded mesenteric rings pre-contracted with S-(-)-Bay K 8644 only at 243, 500 or 750 µg/mL, attenuating the response by rightward shift of the concentration-response curve compared with preparations pre-contracted by KCl 80 mM. These results suggest that EEtOH-ZR probably may acts on vascular smooth muscle by a decrease of calcium influx through Ca\(_V\)L channels only at higher concentrations, as observed on CaCl\(_2\)-induced contractions.

Otherwise, EEtOH-ZR inhibited the vasoconstriction induced by cumulative addition of phenylephrine by a concentration-dependent manner. Phenylephrine promotes increased vascular tone by stimulation of α₁-adrenergic receptor, leading to an increase of two second messengers: inositol 1,4,5-trisphosphate (IP\(_3\)), which promotes the release of calcium from intracellular IP\(_3\)-sensitive calcium stores, and diacylglycerol (DAG), which promotes the activation of contractile proteins and increases calcium influx through voltage-dependent calcium channel. These evidences might explain that EEtOH-ZR possess vasorelaxant effects on voltage-operated calcium channels only at higher concentrations, probably an indirect effect of activation of phenylephrine signaling pathway.

Whereas the inhibition of calcium transmembrane influx probably acts only at highest concentrations, the participation of EEtOH-ZR in lower concentrations on mobilization of calcium intracellular stores was evaluated. After phenylephrine binding to a α₁-receptor, inositol-1,4,5-trisphosphate (IP3) is produced, which binds and activates a IP\(_3\)-specific receptor (IP\(_3\)R) on the sarcoplasmic reticulum (SR) membrane, inducing a Ca\(^{2+}\) internal release and promoting a transient vasoconstriction. Thus, in a Ca\(^{2+}\)-free Tyrode solution, EEtOH-ZR inhibited phenylephrine-induced
transient contractions (10⁻⁵ M) in a concentration-dependent manner, suggesting its possible interference in the calcium mobilization from IP₃-sensitive intracellular stores.

Membrane potential is a key variable that regulates Ca²⁺ influx through voltage-gated Ca²⁺ channels. Further, potassium channels activity importantly contributes to determination and regulation of membrane potential and vascular tone. Actually, potassium channels opening in cell membranes of smooth muscle increases K⁺ efflux, causing membrane hyperpolarization, which constitutes an alternative mechanism of vasodilation of natural products. In order to investigate K⁺ channels participation in the vasorelaxant effect induced by EEtOH-ZR, experiments were performed in presence of tetraethylammonium 3 mM (TEA), a non-selective potassium channel blocker. In the present study, TEA did not attenuate the vasorelaxant effect of EEtOH-ZR in mesenteric artery rings, suggesting that K⁺ channels opening may not be involved in the vasorelaxation of EEtOH-ZR.

In conclusion, the present study demonstrates that the ethanol extract from stem barks of Z. rhoifolium Lam. is capable of modifying the hemodynamic parameters in normotensive and hypertensive rats, promoting decrease of arterial blood pressure probably modulating peripheral vascular resistance by acting on mobilization of calcium intracellular stores.

**Acknowledgment**

This work was supported by UFPI (Federal University of Piauí, Brazil), FAPEPI (Fundação de Amparo à Pesquisa do Estado do Piauí, Brazil) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). There is no conflict of interest associated with the study.

**References**


25 Rocha M L & Bendhack L M, Relaxation evoked by extracellular Ca$^{2+}$ in rat aorta is nerve-independent and involves sarcoplasmic reticulum and L-type Ca$^{2+}$ channel, Vascul Pharmacol, 50 (2009) 98.


28 Zawadlo C & Borlak J, Disease-associated changes in the expression of ion channels, ion receptors, ion exchangers and Ca$^{2+}$-handling proteins in heart hypertrophy, Toxicol Appl Pharmacol, 207 (2005) 244.


